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Surgical Hemotherapy

Symposium organized on the occasion of the 30th anniversary of the Central Laboratory of the Swiss Red Cross, Blood Transfusion Service, and the 10th anniversary of the University Department of Experimental Surgery

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Contents

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Hemotherapy and Antibacterial Defense Mechanisms[1]

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Introduction

Blood has been thought to play a role in infection from the time of antiquity as evidenced by the primitive practice of bloodletting. Later to evolve, but still an old and primitive practice, was the injection of blood into the soft tissues to 'stimulate the bodily defences'. Both techniques were probably of benefit at times since they are now known to be capable of stimulating granulocyte production or release and may create an acute phase reaction associated with elevated levels of blood opsonins. However, despite the relatively enormous advances that have been made in transfusion therapy in the past few decades, the role of blood and blood products in the treatment and prevention of infections continues to be controversial, and modern scientific methodology has not yet been applied to answer even some of the most obvious questions. The three components of the blood which are of potential importance include the red cells, the white cells, and the plasma proteins. Most of the following discussion will be concerned with the components of plasma.

Erythrocyte Transfusions and Infection

It has long been known that anemia may have an association with infection. However, it was soon recognized that most of the adverse effects associated with 'anemia' were a consequence of hypovolemia and/or poor

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nutrition rather than decreased red cell mass. More recent studies have indicated that other things such as plasma volume and leukocyte count being optimal, hematocrit volumes of more than 21% are usually sufficient to support peripheral tissue oxygenation during infection and hematocrit values of 30–35% may be more optimal than values of 40% or more because of a reduction in blood viscosity. Thus, transfusion of red cells solely to correct anemia during infective episodes where the hematocrit is more than 30% is rarely indicated. A much more overwhelming consideration is the blood volume which can more severely restrict peripheral tissue oxygenation. Hypertransfusion has been shown to be beneficial in preventing infection in children with acute lymphocytic leukemia [16], but the effect was probably related more to stimulation of neutrophil production than to elevated red cell mass. Consideration of these aspects is beyond the scope of this presentation, but they are recognized to be critical. They are presented, in part, elsewhere in this symposium.

Leukocyte Transfusions

White cells are absolutely essential for defense against most bacterial pathogens. Lymphocyte transfusions have been reported to have a beneficial effect in certain highly selected conditions such as mucocutaneous candidiasis, but the effects are probably nonspecific and the reports are largely anecdotal. On the other hand, transfusion of neutrophils has been shown to have a definite beneficial effect in many patients who have a transient neutropenia, especially when secondary to cytotoxic agents or irradiation [15]. Such clinical conditions have occurred primarily because of aggressive treatment of malignant disease. The general rule that we have used for indications for leukocyte (neutrophil) transfusions has been the transiently leukopenic patient with neutrophil counts of less than 200/mm^3 for more than 2 days [8] or similar patients who have peripheral neutrophil counts of less than 500–1,000/mm^3 in the face of infection, especially septicemia [1, 10, 11, 17]. To our knowledge, there is no proven benefit of neutrophil transfusions in patients with normal white counts but a dysfunction in neutrophils (e.g. patients with chronic granulomatous disease or severe thermal injury), although this therapy has been advocated by some. There is also no long-term beneficial effect in patients with permanent depression of granulocyte production (e.g. myeloid hypoplasia).

In general, leukocytes collected by continuous flow centrifugation appear

to be functionally better than those obtained by filtration methods, although larger numbers can be obtained by filtration. Because of the short life span of the transfused neutrophils, they should be given daily for several days for the most beneficial effects, and it is generally recognized that neutrophils obtained from HLA identical donors have a longer useful life span [9].

Plasma Therapy

Role of Opsonins and Antibacterial Defense

Several plasma proteins play a critical role in defense against microbial infections, primarily the extracellular bacterial pathogens. These proteins are generally called opsonins and include IgG, IgM, the classical complement components C1, 4, 2, and 3 and the alternative pathway components properdin, factor B and factor D. Less commonly, C5 may play an important role in infections such as those caused by *Candida albicans*. The primary role of these opsonins is via recognition by antibody with deposition of IgG or IgM on the pathogen. Associated with this is activation of either the alternative or classical pathway of complement to generate C3b which is deposited on the microbial surface and the generation of other important mediators which primarily influence the microcirculation causing development of an inflammatory response. Both neutrophils and macrophages have surface receptors for IgG and C3b which provide a means of chemical recognition via attachment to the microbe which results in subsequent ingestion and intracellular killing. These proteins may play other important roles, such as aiding in intracellular digestion, which will not be discussed here. Suffice it to say that the components listed above are absolutely essential for the body's defense against most bacteria [5].

Optimal Concentrations of Opsonins

Very small amounts of antibody which react more or less specifically with the vast majority of potentially pathogenic microbial microbes are present in normal plasma at all times. These small amounts usually trigger activation of the complement sequence to provide opsonization, but a few organisms are opsonized effectively in the apparent absence of specific antibody. The vast majority of organisms are opsonized in a perfectly normal way in serum that is diluted to less than 10% concentration and many organisms are opsonized in 1% serum. A few organisms are more resistant to opsonization and require higher concentrations, up to 50%. Still fewer are

resistant to opsonization in nonimmune serum. The effective concentration of opsonins is therefore difficult to determine except for each individual pathogen, the more pathogenic organisms tending to be more resistant to opsonization. In general, serum opsonic activity of only 10% normal will provide adequate amounts of opsonins for normal killing by normal neutrophils. Experiments on animals using cobra venom factor which will cause consumptive depletion of the alternative pathway of complement have shown that there is not an increased susceptibility to infection with many bacteria unless C3 levels reach less than 10%. Also, experiments in our laboratory using *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* have shown that effective opsonization of bacteria occurs when serum concentrations of C3, factor B and factor D are only 2–4% of normal. Factor B may be the limiting factor in diluted normal serum, but improved opsonization can be obtained by the addition of either C3 or factor B [unpublished data]. Other organisms have not yet been studied.

Concentration of these factors in extracellular fluid, seromas and sites of infection are important considerations *in vivo*. However, little information is available except for normal extracellular fluid which appears to have about 50–75% of serum concentrations for many of the complement components. Opsonic activity decreases progressively with time in seromas, a mechanism which has been proposed as a potential factor in the appearance of late infections in surgical wounds [2]. Presumably, consumptive depletion of opsonic components may be an important factor at localized sites of infection.

Changes in Opsonins with Infection

Previous studies from our laboratory have suggested that bacterial infections can result in the consumption of opsonic proteins which are critical for antibacterial defense, a process we have termed consumptive opsoninopathy [3]. Subsequent studies have indicated that the consumptive opsoninopathy occurs in about 10–15% of bacteremic episodes in high risk surgical patients [6]. However, in the vast majority of infective episodes, there is stimulation of the production of acute phase proteins including C3, factor B and IgG [6]. The overall opsonic index usually changed very little during acute bacteremia, but tended to rise subsequently as a nonspecific response to the infection. Studies in guinea pigs have shown that the rate of recovery of serum opsonic proteins toward normal values is highly dependent upon the nutritional status of the animal after consumptive depletion of the alternative pathway with cobra venom factor [unpublished data]. Thus, it would appear in humans that the reasons for a stimulated

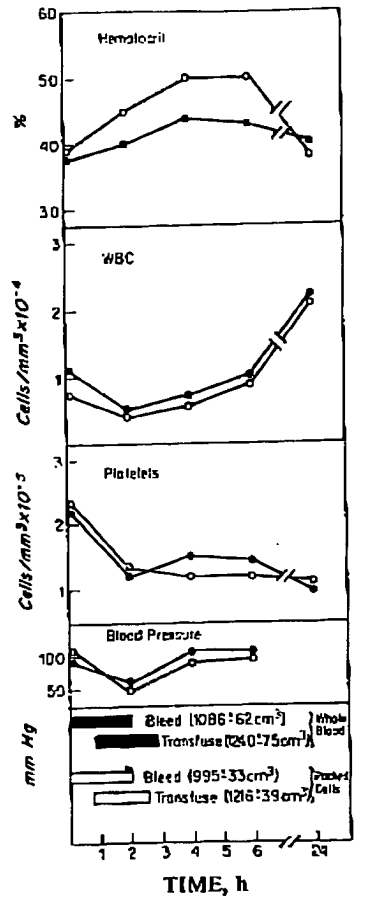
rise in serum opsonic activity and specific proteins in some patients, and a consumptive fall in others is multifactorial and partly dependent on the bacterial challenge, the rate at which the bacteria activate the complement sequence, and the nutritional status of the infected patient.

We conclude from our experiments and the findings of others that acute bacterial infection will often result in a transient depletion of serum opsonic activity which normally stimulates the rapid synthesis of opsonic components to result in greater than normal values. In overwhelming infections, and in nutritionally depleted individuals, the rate of consumption may become greater than the rate of synthesis which may result in fatal outcome without therapeutic intervention.

Changes in Opsonins with Transfusions

A study was performed in conditioned dogs to determine the effect of whole blood compared to packed red cells on the serum levels of opsonic activity and specific components [7]. Animals were bled at a constant rate to remove a volume of blood equal to 8% of the body weight during a 2-hour period. Resuscitation was begun when the systolic arterial pressure reached 60 mm Hg. The rate of infusion initially equalled the bleeding rate but was increased as necessary to maintain systolic arterial pressure at 60 mm Hg. The amount of blood or reconstituted packed cells was equal to the amount shed plus 15%, but an amount equal to an additional 10% of blood volume was given if the arterial pressure did not rise to within 80% of baseline. Additional Ringer's solution was given if a suitable blood pressure was still not achieved (fig. 1). Nearly 100% or better of serum opsonic activity was observed in whole blood recipients at all times in contrast to serum opsonic activity of less than 25% in dogs that received packed cells reconstituted with physiological saline (fig. 2). In dogs treated and resuscitated with packed cells, the C3 levels dropped to 50% of normal as did IgG and total serum protein. Opsonic activity returned to normal by the end of 24 h, but total serum protein, IgG and C3 remained persistently low. There were no significant changes in the animals resuscitated with whole blood. Previous studies in our laboratory have shown that opsonic activity is stable in CPD blood stored under standard conditions at 4°C for as long as 28 days [12].

The conclusions of this study were that a very significant dilutional opsoninopathy may occur in patients with massive blood loss who are resuscitated with packed cells and crystalloid solution and that this can be prevented by resuscitation with whole blood.



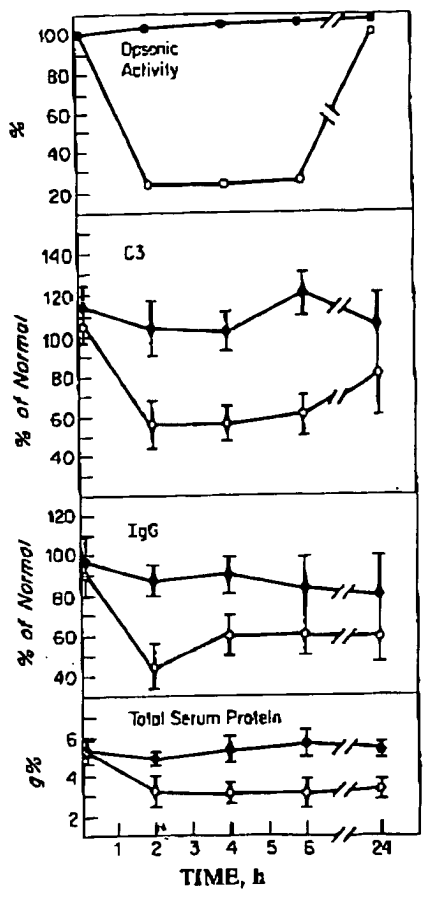


Fig. 1. Physiological effects of hemorrhagic shock and transfusion in dogs. Shown are hematocrit, white blood cell count, platelet count, and blood pressure. O = Dogs receiving packed red cells in saline; ● = dogs receiving whole blood. The period of bleeding and transfusion as well as the volume of fluids administered is shown in the bottom panel. From *Beiting et al.* [7].

Fig. 2. Immunological effects of hemorrhagic shock and transfusion in dogs. Shown are serum opsonic activity (percentage of zero hours), C3 levels (percentage of pooled normal dog serum reference), IgG levels (percentage of normal reference serum), and total serum protein (gram percent). Values shown are the mean ± SE. O = Dogs receiving packed red cells in saline; ● = dogs receiving whole blood. From *Beiting et al.* [7].

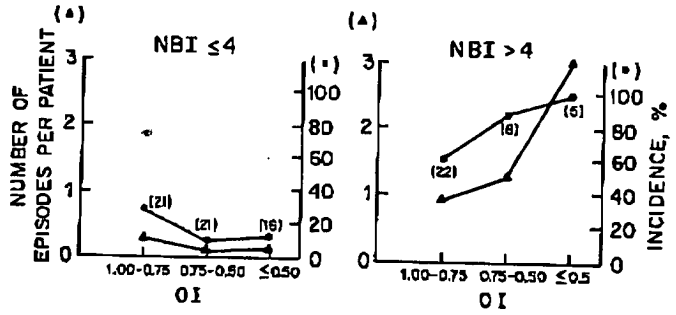


Fig. 3. Relationship of bacteremia and number of bacteremic episodes per patient are related to opsonic activity when grouped into patients with poor neutrophil function [neutrophil bactericidal index (NBI)>4] and patients with normal or mildly abnormal neutrophil function (NBI≤4). Number of patients in parentheses, OI = opsonic index. From *Alexander et al.* [6].

Role of Low Opsonins in Human Infections

We have recently performed a prospective sequential study of the antibacterial function of neutrophils, lymphocyte responsiveness, opsonic capacity of serum and serum levels of C3 (B), properdin, factor B, IgG and albumin in 32 patients with severe burn injury, 21 patients with severe multisystem traumatic injury, 20 patients who were already infected, and 22 patients following renal transplantation [6]. 55 episodes of bacteremia occurred in 37 of these 95 patients. Abnormal neutrophil function was the most important predisposing factor to bacteremia in this study, and the average serum levels of C3, IgG, factor B and properdin had no statistical association with the occurrence of bacteremia. Defective opsonization was associated with a high risk of bacteremia only when there was a coexisting abnormality of neutrophil function (fig. 3). In this study, defective opsonization (less than 75% of normal) appeared to play an important role in predisposition to bacteremia in approximately one third of the bacteremic episodes. The organisms causing bacteremia in these episodes were often relatively resistant to opsonization, in agreement with findings of others [14, 18].

Implications for Therapy

Hereditary deficiencies of IgG, C3, C5 and C3b inactivator have been described which are associated with a high incidence of infection and which respond to therapy with fresh frozen plasma [13]. However, these conditions

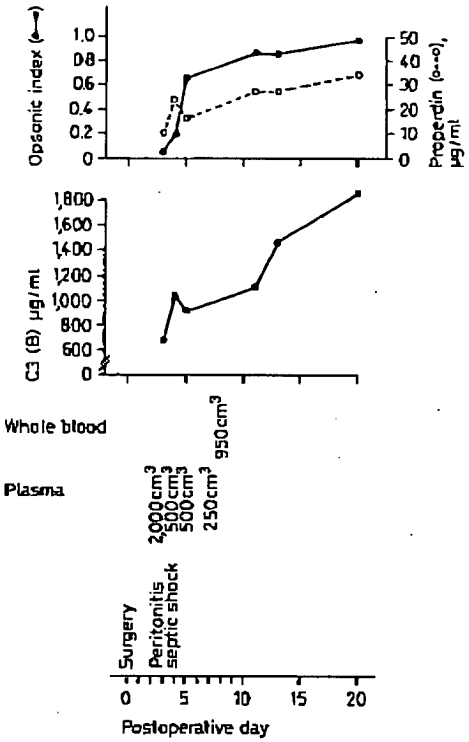


Fig. 4. Serial measurements of C3, properdin and opsonic index in a 31-year-old woman with severe bacterial peritonitis secondary to a ruptured appendix. Fresh frozen plasma in large amounts resulted in restoration of normal values and a marked improvement in her clinical condition.

are rare and of little potential importance compared to acquired deficiencies of opsonic function. Unfortunately, only anecdotal examples currently exist to support vigorous plasma therapy in patients with severe bacterial infections that do not have hereditary defects (fig. 4). However, the low levels of opsonic proteins and function in many patients with persistent severe infections and in severely malnourished patients suggest that intensive plasma therapy may be of benefit.

We have performed a randomized control study to assess the value of prophylactically administered plasma in severely burned patients and have shown that significant improvement in certain of the components can occur with plasma therapy during the initial 2 weeks when the components are

Table I. Bacteremia events in study patients

	Plasma group	Plasma protein derivative group
Number of patients	11	9
Average age, years	22	16
Patients with bacteremia	8	8
Average age, years	22	16
Total bacteremic episodes	10	18
Total number of patient-days at risk[1] for having bacteremia	330	323
Average number of bacteremic episodes per patient-day at risk	0.030	0.062

[1] Patient-days at risk was calculated as the sum of the number of days each patient was in the study minus the number of days in excess of 1 day for bacteremia episodes with a recorded duration greater than 1 day.
From *Alexander et al.* [4].

regularly low, but there is no significant benefit when levels of these components are normal or elevated (i.e. after 2 weeks) [4]. There was no effect on mortality, but the number of bacteremic episodes per patient day of observation was approximately one half in the patients treated with plasma compared to those treated with an equivalent amount of plasma protein derivatives not containing IgG or complement components (table I).

Recommendations

(1) Since opsonic deficiencies appear to contribute to the occurrence of infection in approximately one third of bacteremic patients, it is recommended that whole blood rather than packed cells be used to fulfill transfusion requirements for most patients known to be susceptible to infection, especially when malnourished, and for all patients with severe acute blood loss. Except for certain hereditary deficiencies, it is unlikely that routine plasma therapy will be of benefit to prevent infection in other clinical conditions.

(2) Consideration should be given to the administration of large volumes of fresh frozen plasma (up to 1 liter/day for several days) to patients who have septic shock or to patients with persistent severe infections who fail to respond to appropriate antibiotic and other supportive therapy. Undrained abscesses and endocarditis should first be rigidly excluded in such patients.

(3) A randomized, prospective clinical trial should be performed to determine the precise benefit or lack thereof of routine therapy with fresh frozen plasma in patients with bacteremia or other severe forms of infection. Albumin solutions could be used as an appropriate control.

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Serum Levels of IgG and C3, Postoperative Infections, and Blood Component Therapy

A Prospective Study in 203 Patients

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In 1978, *Beiting et al.* [5] reported that dogs subjected to a controlled blood loss equal to 8% of their body weight, i.e., presumably about 80% of their blood volume, exhibited significant depressions of serum IgG, C3, and opsonic activity when resuscitated with packed red cells in saline. No such changes were observed in control animals resuscitated with whole blood. The salient feature of this experimental study was that serum opsonic activity was impaired at a dilution of IgG and C3 to about 40% of normal levels, i.e., prior to those dilutions of coagulation factors and platelets which are known to require the use of fresh frozen plasma and/or fresh whole blood anyway in the bleeding patient. If confirmed in the clinical setting, these findings would therefore be important for the use of blood component therapy in surgical patients. Since blood stored under standard conditions retains an almost normal opsonic activity against various pathogenic microorganisms [8, 9], the potential problem is the plasma deficit of red cell concentrates as compared to whole blood, as also suggested by *Beiting et al.* [5].

At variance with their results, studies in patients with a congenital C3 deficiency have shown that serum C3 levels must be as low as 10% or less of normal to generate a clinically increased susceptibility to infections [1–3]. *Weinstein and Young* [12], furthermore, found that serum C3 levels and opsonic activity do not necessarily correlate, and that the same plasma may have different opsonic activities against an infecting and laboratory bacterial strains. As to IgG, the usually accepted threshold for clinically significant 'hypogammaglobulinemia' is 200 mg/dl according to the study of the British Medical Research Council [10], or 20–25% of the normal levels. The evidence for a relationship between serum IgG and C3 levels and antibac-